

In re Application of: Pietrokovski et al
Serial No.: 10/534,544
Filed: May 10, 2005
Office Action Mailing Date: May 12, 2008

Examiner: Ogunbiyi
Group Art Unit: 1645
Attorney Docket: 29489

In the Claims:

1. (Currently Amended) A chimeric polypeptide comprising an autoprocessing segment having ~~an~~^{the} amino acid sequence set forth by SEQ ID NO: 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105 or 106.

2-4. (Cancelled)

5. (Currently Amended) The chimeric polypeptide of claim 1, wherein a carboxy terminal end of said autoprocessing segment is attached to a first polypeptide segment and further wherein said auto-cleavageprocessing results in removal of a said first polypeptide segment of the polypeptide adjacent to an amino terminal end or a carboxy terminal end of said autoprocessing segment.

6. (Currently Amended) The chimeric polypeptide of claim 51, wherein said autoprocessing segment is flanked by a first and a second polypeptide segments and further wherein said autoprocessing results in splicing of said first and said second polypeptide segments ~~segment of the polypeptide adjacent to said autoprocessing segment is an amino terminal segment or a carboxy terminal segment of the polypeptide.~~

7. (Original) The chimeric polypeptide of claim 5, wherein said segment of the polypeptide adjacent to said carboxy terminal end of said

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autoprocessing segment includes an amino acid residue comprising a nucleophilic group at an amino terminal end thereof.

8. (Original) The chimeric polypeptide of claim 7, wherein said nucleophilic group is a hydroxyl group.

9. (Original) The chimeric polypeptide of claim 7, wherein said amino acid residue is a threonine residue.

10. (Original) The chimeric polypeptide of claim 5, wherein said segment of the polypeptide adjacent to said amino terminal end of said autoprocessing segment includes a serine amino acid residue at a carboxy terminal end thereof.

11. (Currently Amended) The chimeric polypeptide of claim 1, wherein said auto-cleavageprocessing results in auto-splicing.

12. (Original) The chimeric polypeptide of claim 11, wherein said auto-splicing is auto-splicing of segments of the polypeptide flanking said autoprocessing segment.

13. (Currently Amended) The chimeric polypeptide of claim 1, wherein the chimeric polypeptide is capable of active in said auto-cleavageprocessing under a condition selected from the group consisting of a temperature selected from a range of 33 °C to 41 °C, a pH selected from a range of pH 7.8 to pH 8.2, and a concentration of dithiothreitol selected from a range of 0.1 mM to 20 mM.

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14. (Currently Amended) The chimeric polypeptide of claim 1, further comprising an affinity tag capable of specifically binding a substratemolecule.

15. (Original) The chimeric polypeptide of claim 14, wherein said affinity tag is a maltose-binding domain or a chitin-binding domain.

16. (Currently Amended) The chimeric polypeptide of claim 14, wherein said ~~substrate is selected from the group consisting of a molecule, a compound, a molecule forms a part of a virus, and or a cell.~~

17. (Currently Amended) The chimeric polypeptide of claim 146, wherein said molecule is amylose or chitin.

18. (Original) The chimeric polypeptide of claim 16, wherein said virus is a bacteriophage.

19. (Withdrawn) A polynucleotide encoding a chimeric polypeptide comprising an autoprocessing segment having an amino acid sequence set forth by SEQ ID NO: 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105 or 106, the polypeptide being capable of auto-cleavage.

20. (Withdrawn) The polynucleotide of claim 19, wherein said autoprocessing segment is selected from the group consisting of BIL1_cloth, BIL2_cloth, BIL3_cloth, BIL4_cloth, BIL5_cloth, BIL6_cloth, BIL7_cloth,

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BIL9_cloth, BIL10_cloth, BIL11_cloth, 3875_87_magma, FhaB_manha, BIL2_neigo, BIL3_neigo, BIL5_neigo, BIL6_neigo, MafB1_neigo, MafB2_neigo, B0369+_neimeB, B0372+_neimeB, B0655+_neimeB, A2115_neime, BIL2_neimeC, BIL3_neimeC, BIL4_neimeC, BIL5_neimeC, BIL6_neimeC, MafB1_neimeC, FhaB1_psefl-PfO-1, FhaB1_psefl-SBW25, FhaB_psesy, SCP1.201_strco, 39_9_thefus, BIL1_gemob, BIL2_gemob, 0709_lepin, 3725_lepin, 3719_lepin, o665_myxxa, o1078_myxxa, o1070_myxxa, BIL1_strav, BIL2_strav, BIL3_strav, BIL1_pirsp, BIL1_chrvi, BIL1_glovi, BIL2_glovi, BIL3_glovi, BIL4_glovi, BIL5_glovi, BIL6_glovi, BIL7_glovi, o649_versp, o5687_versp, o3395_versp, II0519_brume, BIL2_magma, BIL3_magma, BIL4_magma, BIL5_magma, BIL6_magma, 06786_metex, 00126_rhoca, 00199_rhoca, 00459_rhoca, 00460_rhoca, 00746_rhoca, 00949_rhoca, 01216_rhoca, 01374_rhoca, 01523_rhoca, 01524_rhoca, 02710_rhoca, 03530_rhoca, 4825_rhosp, BIL2_rhosp, BIL1_silpo, BIL2_silpo, BIL3_silpo, BIL4_silpo, BIL5_silpo, BIL6_silpo, BIL7_silpo, BIL8_silpo, BIL9_silpo, BIL10_silpo, BIL11_silpo, BIL12_silpo, BIL13_silpo, BIL14_silpo, BIL15_silpo, BIL16_silpo, Bill1_rhile, BIL1_unknwn, and BIL2_unknwn.

21. (Withdrawn) The polynucleotide of claim 19, wherein said autoprocessing segment is derived from a protein of an organism belonging to a genus selected from the group consisting of *Brucella*, *Clostridium*, *Magnetospirillum*, *Mannheimia*, *Methylobacterium*, *Neisseria*, *Pseudomonas*, *Rhodobacter*, *Silicibacter*, *Streptomyces*, *Thermobifida*, *Rhizobium*, *Chromobacterium*, *Myxococcus*, *Leptospira*, *Pirellula*, *Gemmata*, *Gloeobacter* and *Verrucomicrobium*.

22. (Withdrawn) The polynucleotide of claim 21, wherein said organism is selected from the group consisting of *Rhodobacter capsulatus*, *Rhodobacter*

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sphaeroides, *Silicibacter pomeroyi*, *Brucella melitensis*, *Brucella suis*, *Magnetospirillum magnetotacticum*, *Methylobacterium extorquens*, *Rhizobium leguminosarum*, *Neisseria meningitidis*, *Neisseria meningitidis*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Chromobacterium violaceum*, *Pseudomonas syringae*, *Pseudomonas fluorescens*, *Pseudomonas fluorescens*, *Mannheimia haemolytica*, *Myxococcus xanthus*, *Leptospira interrogans*, *Streptomyces coelicolor*, *Streptomyces avermitilis*, *Thermobifida fusca*, *Clostridium thermocellum*, *Pirellula species 1*, *Gemmata obscuriglobus*, *Gloeobacter violaceus*, and *Verrucomicrobium spinosum*.

23. (Withdrawn) The polynucleotide of claim 19, wherein said autocleavage results in removal of a segment of the chimeric polypeptide adjacent to an amino terminal end or a carboxy terminal end of said autoprocessing segment.

24. (Withdrawn) The polynucleotide of claim 23, wherein said segment of the chimeric polypeptide adjacent to said autoprocessing segment is an amino terminal segment or a carboxy terminal segment of the chimeric polypeptide.

25. (Withdrawn) The polynucleotide of claim 23, wherein said segment of the chimeric polypeptide adjacent to said carboxy terminal end of said autoprocessing segment includes an amino acid residue comprising a nucleophilic group at an amino terminal end thereof.

26. (Withdrawn) The polynucleotide of claim 25, wherein said nucleophilic group is a hydroxyl group.

27. (Withdrawn) The polynucleotide of claim 25, wherein said amino acid residue is a threonine residue.

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28. (Withdrawn) The polynucleotide of claim 23, wherein said segment of the chimeric polypeptide adjacent to said amino terminal end of said autoprocessing segment includes a serine amino acid residue at a carboxy terminal end thereof.

29. (Withdrawn) The polynucleotide of claim 19, wherein said auto-cleavage results in auto-splicing.

30. (Withdrawn) The polynucleotide of claim 29, wherein said auto-splicing is auto-splicing of segments of the chimeric polypeptide flanking said autoprocessing segment.

31. (Withdrawn) The polynucleotide of claim 19, wherein the chimeric polypeptide is capable of said auto-cleavage under a condition selected from the group consisting of a temperature selected from a range of 33 °C to 41 °C, a pH selected from a range of pH 7.8 to pH 8.2, and a concentration of dithiothreitol selected from a range of 0.1 mM to 20 mM.

32. (Withdrawn) The polynucleotide of claim 19, wherein the chimeric polypeptide further comprises an affinity tag capable of specifically binding a substrate.

33. (Withdrawn) The polynucleotide of claim 32, wherein said affinity tag is a maltose-binding domain or a chitin-binding domain.

34. (Withdrawn) The polynucleotide of claim 32, wherein said substrate is selected from the group consisting of a molecule, a compound, a virus, and a cell.

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35. (Withdrawn) The polynucleotide of claim 33, wherein said molecule is amylose or chitin.

36. (Withdrawn) The polynucleotide of claim 33, wherein said virus is a bacteriophage.

37. (Withdrawn) The polynucleotide of claim 19, further comprising a promoter sequence being for directing expression of the chimeric polypeptide in an expression system.

38. (Withdrawn) The polynucleotide of claim 37, wherein said expression system is a cellular expression system or a cell-free expression system.

39. (Withdrawn) The polynucleotide of claim 38, wherein said cellular expression system is an *E. coli* cellular expression system.

40. (Withdrawn) The polynucleotide of claim 38, wherein said cell-free expression system is an *E. coli* S30 extract expression system.

41. (Withdrawn) The polynucleotide of claim 37, wherein said promoter sequence is inducible by isopropyl beta-D-thiogalactoside.

42. (Withdrawn) A nucleic acid construct comprising a nucleic acid sequence encoding a chimeric polypeptide comprising an autoprocessing segment having an amino acid sequence set forth by SEQ ID NO: 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61,

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62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84,
85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105
or 106, said chimeric polypeptide being capable of auto-cleavage.

43. (Withdrawn) The nucleic acid construct of claim 42, further comprising a promoter sequence being for directing expression of said chimeric polypeptide in an expression system.

44. (Withdrawn) The nucleic acid construct of claim 43, wherein said expression system is a cellular expression system or a cell-free expression system.

45. (Withdrawn) The nucleic acid construct of claim 44, wherein said cellular expression system is an *E. coli* cellular expression system.

46. (Withdrawn) The nucleic acid construct of claim 44, wherein said cell-free expression system is an *E. coli* S30 extract expression system.

47. (Withdrawn) The nucleic acid construct of claim 43, wherein said promoter sequence is inducible by isopropyl beta-D-thiogalactoside.

48. (Withdrawn) The nucleic acid construct of claim 42, wherein said autoprocessing segment is selected from the group consisting of BIL1_cloth, BIL2_cloth, BIL3_cloth, BIL4_cloth, BIL5_cloth, BIL6_cloth, BIL7_cloth, BIL9_cloth, BIL10_cloth, BIL11_cloth, 3875_87_magma, FhaB_manha, BIL2_neigo, BIL3_neigo, BIL5_neigo, BIL6_neigo, MafB1_neigo, MafB2_neigo, B0369+_neimeB, B0372+_neimeB, B0655+_neimeB, A2115_neime, BIL2_neimeC, BIL3_neimeC, BIL4_neimeC, BIL5_neimeC, BIL6_neimeC, MafB1_neimeC, FhaB1_psefl-PfO-1, FhaB1_psefl-SBW25, FhaB_psesy, SCP1.201_strco,

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39_9_thefus, BIL1_gemob, BIL2_gemob, 0709_lepin, 3725_lepin, 3719_lepin, o665_myxxa, o1078_myxxa, o1070_myxxa, BIL1_strav, BIL2_strav, BIL3_strav, BIL1_pirsp, BIL1_chrvi, BIL1_glovi, BIL2_glovi, BIL3_glovi, BIL4_glovi, BIL5_glovi, BIL6_glovi, BIL7_glovi, o649_versp, o5687_versp, o3395_versp, II0519_brume, BIL2_magma, BIL3_magma, BIL4_magma, BIL5_magma, BIL6_magma, 06786_metex, 00126_rhoca, 00199_rhoca, 00459_rhoca, 00460_rhoca, 00746_rhoca, 00949_rhoca, 01216_rhoca, 01374_rhoca, 01523_rhoca, 01524_rhoca, 02710_rhoca, 03530_rhoca, 4825_rhosp, BIL2_rhosp, BIL1_silpo, BIL2_silpo, BIL3_silpo, BIL4_silpo, BIL5_silpo, BIL6_silpo, BIL7_silpo, BIL8_silpo, BIL9_silpo, BIL10_silpo, BIL11_silpo, BIL12_silpo, BIL13_silpo, BIL14_silpo, BIL15_silpo, BIL16_silpo, Bil1_rhile, BIL1_unknwn, and BIL2_unknwn.

49. (Withdrawn) The nucleic acid construct of claim 42, wherein said autoprocessing segment is derived from a protein of an organism belonging to a genus selected from the group consisting of *Brucella*, *Clostridium*, *Magnetospirillum*, *Mannheimia*, *Methylobacterium*, *Neisseria*, *Pseudomonas*, *Rhodobacter*, *Silicibacter*, *Streptomyces*, *Thermobifida*, *Rhizobium*, *Chromobacterium*, *Myxococcus*, *Leptospira*, *Pirellula*, *Gemmata*, *Gloeobacter* and *Verrucomicrobium*.

50. (Withdrawn) The nucleic acid construct of claim 49, wherein said organism is selected from the group consisting of *Rhodobacter capsulatus*, *Rhodobacter sphaeroides*, *Silicibacter pomeroyi*, *Brucella melitensis*, *Brucella suis*, *Magnetospirillum magnetotacticum*, *Methylobacterium extorquens*, *Rhizobium leguminosarum*, *Neisseria meningitidis*, *Neisseria meningitidis*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Chromobacterium violaceum*, *Pseudomonas syringae*, *Pseudomonas fluorescens*, *Pseudomonas fluorescens*, *Mannheimia*

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haemolytica, Myxococcus xanthus, Leptospira interrogans, Streptomyces coelicolor, Streptomyces avermitilis, Thermobifida fusca, Clostridium thermocellum, Pirellula species 1, Gemmata obscuriglobus, Gloeobacter violaceus, and Verrucomicrobium spinosum.

51. (Withdrawn) The nucleic acid construct of claim 42, wherein said auto-cleavage results in removal of a segment of said chimeric polypeptide adjacent to an amino terminal end or a carboxy terminal end of said autoprocessing segment.

52. (Withdrawn) The nucleic acid construct of claim 51, wherein said segment of said chimeric polypeptide adjacent to said autoprocessing segment is an amino terminal segment or a carboxy terminal segment of said chimeric polypeptide.

53. (Withdrawn) The nucleic acid construct of claim 51, wherein said segment of said chimeric polypeptide adjacent to said carboxy terminal end of said autoprocessing segment includes an amino acid residue comprising a nucleophilic group at an amino terminal end thereof.

54. (Withdrawn) The nucleic acid construct of claim 53, wherein said nucleophilic group is a hydroxyl group.

55. (Withdrawn) The nucleic acid construct of claim 53, wherein said amino acid residue is a threonine residue.

56. (Withdrawn) The nucleic acid construct of claim 51, wherein said segment of said chimeric polypeptide adjacent to said amino terminal end of said autoprocessing segment includes a serine amino acid residue at a carboxy terminal end thereof.

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57. (Withdrawn) The nucleic acid construct of claim 42, wherein said auto-cleavage results in auto-splicing.

58. (Withdrawn) The nucleic acid construct of claim 57, wherein said auto-splicing is auto-splicing of segments of said chimeric polypeptide flanking said autoprocessing segment.

59. (Withdrawn) The nucleic acid construct of claim 42, wherein said chimeric polypeptide is capable of said auto-cleavage under a condition selected from the group consisting of a temperature selected from a range of 33 °C to 41 °C, a pH selected from a range of pH 7.8 to pH 8.2, and a concentration of dithiothreitol selected from a range of 0.1 mM to 20 mM.

60. (Withdrawn) The nucleic acid construct of claim 42, wherein said chimeric polypeptide further comprises an affinity tag capable of specifically binding a substrate.

61. (Withdrawn) The nucleic acid construct of claim 60, wherein said affinity tag is a maltose-binding domain or a chitin-binding domain.

62. (Withdrawn) A method of generating a chimeric polypeptide capable of displaying auto-cleavage, the method comprising generating a chimeric amino acid sequence including an autoprocessing segment, said autoprocessing segment having an amino acid sequence set forth by SEQ ID NO: 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85,

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86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105 or 106, thereby producing the chimeric polypeptide capable of displaying auto-cleavage.

63. (Withdrawn) The method of claim 62, wherein said generating said chimeric amino acid sequence is effected by synthesizing a polynucleotide encoding said chimeric amino acid sequence and expressing said polynucleotide in an expression system.

64. (Withdrawn) The method of claim 63, wherein said expression system is a cellular expression system or a cell-free expression system.

65. (Withdrawn) The method of claim 64, wherein said cellular expression system is an *E. coli* cellular expression system.

66. (Withdrawn) The method of claim 64, wherein said cell-free expression system is an *E. coli* S30 extract expression system.

67. (Withdrawn) The method of claim 63, wherein said polynucleotide comprises a promoter sequence being for directing said expression of the chimeric polypeptide.

68. (Withdrawn) The method of claim 67, wherein said promoter sequence is inducible by isopropyl beta-D-thiogalactoside.

69. (Withdrawn) The method of claim 62, wherein said autoprocessing segment is selected from the group consisting of BIL1_cloth, BIL2_cloth, BIL3_cloth, BIL4_cloth, BIL5_cloth, BIL6_cloth, BIL7_cloth, BIL9_cloth, BIL10_cloth, BIL11_cloth, 3875_87_magma, FhaB_manha, BIL2_neigo,

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BIL3_neigo, BIL5_neigo, BIL6_neigo, MafB1_neigo, MafB2_neigo, B0369+_neimeB, B0372+_neimeB, B0655+_neimeB, A2115_neime, BIL2_neimeC, BIL3_neimeC, BIL4_neimeC, BIL5_neimeC, BIL6_neimeC, MafB1_neimeC, FhaB1_psefl-PfO-1, FhaB1_psefl-SBW25, FhaB_psesy, SCP1.201_strco, 39_9_thefus, BIL1_gemob, BIL2_gemob, 0709_lepin, 3725_lepin, 3719_lepin, o665_myxxa, o1078_myxxa, o1070_myxxa, BIL1_strav, BIL2_strav, BIL3_strav, BIL1_pirsp, BIL1_chrvi, BIL1_glovi, BIL2_glovi, BIL3_glovi, BIL4_glovi, BIL5_glovi, BIL6_glovi, BIL7_glovi, o649_versp, o5687_versp, o3395_versp, II0519_brume, BIL2_magma, BIL3_magma, BIL4_magma, BIL5_magma, BIL6_magma, 06786_metex, 00126_rhoca, 00199_rhoca, 00459_rhoca, 00460_rhoca, 00746_rhoca, 00949_rhoca, 01216_rhoca, 01374_rhoca, 01523_rhoca, 01524_rhoca, 02710_rhoca, 03530_rhoca, 4825_rhosp, BIL2_rhosp, BIL1_silpo, BIL2_silpo, BIL3_silpo, BIL4_silpo, BIL5_silpo, BIL6_silpo, BIL7_silpo, BIL8_silpo, BIL9_silpo, BIL10_silpo, BIL11_silpo, BIL12_silpo, BIL13_silpo, BIL14_silpo, BIL15_silpo, BIL16_silpo, Bil1_rhile, BIL1_unknwn, and BIL2_unknwn.

70. (Withdrawn) The method of claim 62, wherein said autoproCESSing segment is derived from a protein of an organism belonging to a genus selected from the group consisting of *Brucella*, *Clostridium*, *Magnetospirillum*, *Mannheimia*, *Methylobacterium*, *Neisseria*, *Pseudomonas*, *Rhodobacter*, *Silicibacter*, *Streptomyces*, *Thermobifida*, *Rhizobium*, *Chromobacterium*, *Myxococcus*, *Leptospira*, *Pirellula*, *Gemmata*, *Gloeobacter* and *Verrucomicrobium*.

71. (Withdrawn) The method of claim 70, wherein said organism is selected from the group consisting of *Rhodobacter capsulatus*, *Rhodobacter sphaeroides*, *Silicibacter pomeroyi*, *Brucella melitensis*, *Brucella suis*, *Magnetospirillum magnetotacticum*, *Methylobacterium extorquens*, *Rhizobium*

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leguminosarum, *Neisseria meningitidis*, *Neisseria meningitidis*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Chromobacterium violaceum*, *Pseudomonas syringae*, *Pseudomonas fluorescens*, *Pseudomonas fluorescens*, *Mannheimia haemolytica*, *Myxococcus xanthus*, *Leptospira interrogans*, *Streptomyces coelicolor*, *Streptomyces avermitilis*, *Thermobifida fusca*, *Clostridium thermocellum*, *Pirellula species 1*, *Gemmata obscuriglobus*, *Gloeobacter violaceus*, and *Verrucomicrobium spinosum*.

72. (Withdrawn) The method of claim 62, wherein said auto-cleavage results in removal of a segment of the chimeric polypeptide adjacent to an amino terminal end or a carboxy terminal end of said autoprocessing segment.

73. (Withdrawn) The method of claim 72, wherein said segment of the chimeric polypeptide adjacent to said autoprocessing segment is an amino terminal segment or a carboxy terminal segment of the chimeric polypeptide.

74. (Withdrawn) The method of claim 72, wherein said segment of the chimeric polypeptide adjacent to said carboxy terminal end of said autoprocessing segment includes an amino acid residue comprising a nucleophilic group at an amino terminal end thereof.

75. (Withdrawn) The method of claim 74, wherein said nucleophilic group is a hydroxyl group.

76. (Withdrawn) The method of claim 74, wherein said amino acid residue is a threonine residue.

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77. (Withdrawn) The method of claim 72, wherein said segment of the chimeric polypeptide adjacent to said amino terminal end of said autoprocessing segment includes a serine amino acid residue at a carboxy terminal end thereof.

78. (Withdrawn) The method of claim 62, wherein said auto-cleavage results in auto-splicing.

79. (Withdrawn) The method of claim 78, wherein said auto-splicing is auto-splicing of segments of the chimeric polypeptide flanking said autoprocessing segment.

80. (Withdrawn) The method of claim 62, wherein the chimeric polypeptide is capable of said auto-cleavage under a condition selected from the group consisting of a temperature selected from a range of 33 °C to 41 °C, a pH selected from a range of pH 7.8 to pH 8.2, and a concentration of dithiothreitol selected from a range of 0.1 mM to 20 mM.

81. (Withdrawn) The method of claim 62, wherein the chimeric polypeptide includes an affinity tag.

82. (Withdrawn) The method of claim 81, wherein said affinity tag is a maltose-binding domain or a chitin-binding domain.

83. (Withdrawn) A method of purifying a protein, the method comprising:

- (a) generating a chimeric polypeptide including an autoprocessing segment having an amino acid sequence set forth by SEQ ID NO: 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28,

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29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47,
48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65,
66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84,
85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102,
103, 104, 105 or 106, said autoprocessing segment being terminally
attached to, or flanked by, an amino acid sequence of the protein, said
chimeric polypeptide being capable of auto-cleavage when subjected
to suitable conditions to thereby remove said amino acid sequence of
the protein from said chimeric polypeptide thereby generating the
protein;

- (b) immobilizing said chimeric polypeptide to a support; and
- (c) subjecting said chimeric polypeptide to said suitable conditions,
thereby purifying the protein.

84. (Withdrawn) The method of claim 83, further comprising the step of
separating the protein from said autoprocessing segment following step (c).

85. (Withdrawn) The method of claim 83, wherein said support includes
an antibody or antibody fragment capable of specifically binding said autoprocessing
segment, and whereas said immobilizing is via said autoprocessing segment.

86. (Withdrawn) The method of claim 83, wherein said chimeric
polypeptide further includes an affinity tag sequence, and whereas said immobilizing
is via said affinity tag sequence.

87. (Withdrawn) The method of claim 86, wherein said support includes
a specific ligand of said affinity tag sequence, and whereas said immobilizing is via
said specific ligand of said affinity tag sequence.

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88. (Withdrawn) The method of claim 83, wherein said generating said chimeric polypeptide is effected by synthesizing a polynucleotide encoding said chimeric polypeptide and expressing said polynucleotide in an expression system.

89. (Withdrawn) The method of claim 88, wherein said expression system is a cellular expression system or a cell-free expression system.

90. (Withdrawn) The method of claim 89, wherein said cellular expression system is an *E. coli* cellular expression system.

91. (Withdrawn) The method of claim 89, wherein said cell-free expression system is an *E. coli* S30 extract expression system.

92. (Withdrawn) The method of claim 88, wherein said polynucleotide comprises a promoter sequence being for directing said expression of said chimeric polypeptide.

93. (Withdrawn) The method of claim 92, wherein said promoter sequence is inducible by isopropyl beta-D-thiogalactoside.

94. (Withdrawn) The method of claim 83, wherein said autoprocessing segment is selected from the group consisting of BIL1_cloth, BIL2_cloth, BIL3_cloth, BIL4_cloth, BIL5_cloth, BIL6_cloth, BIL7_cloth, BIL9_cloth, BIL10_cloth, BIL11_cloth, 3875_87_magma, FhaB_manha, BIL2_neigo, BIL3_neigo, BIL5_neigo, BIL6_neigo, MafB1_neigo, MafB2_neigo, B0369+_neimeB, B0372+_neimeB, B0655+_neimeB, A2115_neime, BIL2_neimeC, BIL3_neimeC, BIL4_neimeC, BIL5_neimeC, BIL6_neimeC, MafB1_neimeC,

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FhaB1_psefl-PfO-1, FhaB1_psefl-SBW25, FhaB_psesy, SCP1.201_strco, 39_9_thefus, BIL1_gemob, BIL2_gemob, 0709_lepin, 3725_lepin, 3719_lepin, o665_myxxa, o1078_myxxa, o1070_myxxa, BIL1_strav, BIL2_strav, BIL3_strav, BIL1_pirsp, BIL1_chrvi, BIL1_glovi, BIL2_glovi, BIL3_glovi, BIL4_glovi, BIL5_glovi, BIL6_glovi, BIL7_glovi, o649_versp, o5687_versp, o3395_versp, II0519_brume, BIL2_magma, BIL3_magma, BIL4_magma, BIL5_magma, BIL6_magma, 06786_metex, 00126_rhoca, 00199_rhoca, 00459_rhoca, 00460_rhoca, 00746_rhoca, 00949_rhoca, 01216_rhoca, 01374_rhoca, 01523_rhoca, 01524_rhoca, 02710_rhoca, 03530_rhoca, 4825_rhosp, BIL2_rhosp, BIL1_silpo, BIL2_silpo, BIL3_silpo, BIL4_silpo, BIL5_silpo, BIL6_silpo, BIL7_silpo, BIL8_silpo, BIL9_silpo, BIL10_silpo, BIL11_silpo, BIL12_silpo, BIL13_silpo, BIL14_silpo, BIL15_silpo, BIL16_silpo, Bill1_rhile, BIL1_unknwn, and BIL2_unknwn.

95. (Withdrawn) The method of claim 83, wherein said autoprocessing segment is derived from a protein of an organism belonging to a genus selected from the group consisting of *Brucella*, *Clostridium*, *Magnetospirillum*, *Mannheimia*, *Methylobacterium*, *Neisseria*, *Pseudomonas*, *Rhodobacter*, *Silicibacter*, *Streptomyces*, *Thermobifida*, *Rhizobium*, *Chromobacterium*, *Myxococcus*, *Leptospira*, *Pirellula*, *Gemmata*, *Gloeobacter* and *Verrucomicrobium*.

96. (Withdrawn) The method of claim 95, wherein said organism is selected from the group consisting of *Rhodobacter capsulatus*, *Rhodobacter sphaeroides*, *Silicibacter pomeroyi*, *Brucella melitensis*, *Brucella suis*, *Magnetospirillum magnetotacticum*, *Methylobacterium extorquens*, *Rhizobium leguminosarum*, *Neisseria meningitidis*, *Neisseria meningitidis*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Chromobacterium violaceum*, *Pseudomonas syringae*, *Pseudomonas fluorescens*, *Pseudomonas fluorescens*, *Mannheimia*

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haemolytica, Myxococcus xanthus, Leptospira interrogans, Streptomyces coelicolor, Streptomyces avermitilis, Thermobifida fusca, Clostridium thermocellum, Pirellula species 1, Gemmata obscuriglobus, Gloeobacter violaceus, and Verrucomicrobium spinosum.

97. (Withdrawn) The method of claim 83, wherein said auto-cleavage results in removal of a segment of said chimeric polypeptide adjacent to an amino terminal end or a carboxy terminal end of said autoprocessing segment.

98. (Withdrawn) The method of claim 97, wherein said segment of said chimeric polypeptide adjacent to said autoprocessing segment is an amino terminal segment or a carboxy terminal segment of said chimeric polypeptide.

99. (Withdrawn) The method of claim 97, wherein said segment of said chimeric polypeptide adjacent to said carboxy terminal end of said autoprocessing segment includes an amino acid residue comprising a nucleophilic group at an amino terminal end thereof.

100. (Withdrawn) The method of claim 99, wherein said nucleophilic group is a hydroxyl group.

101. (Withdrawn) The method of claim 99, wherein said amino acid residue is a threonine residue.

102. (Withdrawn) The method of claim 97, wherein said segment of said chimeric polypeptide adjacent to said amino terminal end of said autoprocessing segment includes a serine amino acid residue at a carboxy terminal end thereof.

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103. (Withdrawn) The method of claim 83, wherein said auto-cleavage results in auto-splicing.

104. (Withdrawn) The method of claim 103, wherein said auto-splicing is auto-splicing of segments of said chimeric polypeptide flanking said autoprocessing segment.

105. (Withdrawn) The method of claim 83, wherein said chimeric polypeptide is capable of said auto-cleavage under a condition selected from the group consisting of a temperature selected from a range of 33 °C to 41 °C, a pH selected from a range of pH 7.8 to pH 8.2, and a concentration of dithiothreitol selected from a range of 0.1 mM to 20 mM.

106. (Withdrawn) A method of reversibly attaching a first substrate to a second substrate, the method comprising:

- (a) providing a chimeric polypeptide including an autoprocessing segment having an amino acid sequence set forth by SEQ ID NO: 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105 or 106 flanked by a first amino acid sequence capable of binding the first substrate and a second amino acid sequence capable of binding the second substrate, said chimeric polypeptide being capable of auto-cleavage when subjected to suitable conditions, to thereby release said first amino acid sequence from said second amino acid sequence;

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- (b) exposing the first substrate and the second substrate to said chimeric polypeptide, thereby generating a complex including the first substrate attached via said chimeric polypeptide to the second substrate; and
- (c) subjecting said complex to said suitable conditions, thereby detaching the first substrate from the second substrate.

107. (Withdrawn) The method of claim 106, wherein each of the first and second substrates is independently selected from the group consisting of a molecule, a compound, a virus, and a cell.

108. (Withdrawn) The method of claim 107, wherein said molecule is amylose or chitin.

109. (Withdrawn) The method of claim 107, wherein said virus is a bacteriophage.

110. (Withdrawn) The method of claim 106, wherein said chimeric polypeptide includes an affinity tag sequence, and whereas said binding the first substrate or said binding the second substrate is via said affinity tag sequence.

111. (Withdrawn) The method of claim 110, wherein said affinity tag sequence is a maltose-binding domain or a chitin-binding domain.

112. (Withdrawn) The method of claim 106, wherein said autoprocessing segment is selected from the group consisting of BIL1_cloth, BIL2_cloth, BIL3_cloth, BIL4_cloth, BIL5_cloth, BIL6_cloth, BIL7_cloth, BIL9_cloth, BIL10_cloth, BIL11_cloth, 3875_87_magma, FhaB_manha, BIL2_neigo, BIL3_neigo, BIL5_neigo, BIL6_neigo, MafB1_neigo, MafB2_neigo,

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B0369+_neimeB, B0372+_neimeB, B0655+_neimeB, A2115_neime, BIL2_neimeC, BIL3_neimeC, BIL4_neimeC, BIL5_neimeC, BIL6_neimeC, MafB1_neimeC, FhaB1_psefl-PfO-1, FhaB1_psefl-SBW25, FhaB_psesy, SCP1.201_strco, 39_9_thefus, BIL1_gemob, BIL2_gemob, 0709_lepin, 3725_lepin, 3719_lepin, o665_myxxa, o1078_myxxa, o1070_myxxa, BIL1_strav, BIL2_strav, BIL3_strav, BIL1_pirsp, BIL1_chrvi, BIL1_glovi, BIL2_glovi, BIL3_glovi, BIL4_glovi, BIL5_glovi, BIL6_glovi, BIL7_glovi, o649_versp, o5687_versp, o3395_versp, II0519_brume, BIL2_magma, BIL3_magma, BIL4_magma, BIL5_magma, BIL6_magma, 06786_metex, 00126_rhoca, 00199_rhoca, 00459_rhoca, 00460_rhoca, 00746_rhoca, 00949_rhoca, 01216_rhoca, 01374_rhoca, 01523_rhoca, 01524_rhoca, 02710_rhoca, 03530_rhoca, 4825_rhosp, BIL2_rhosp, BIL1_silpo, BIL2_silpo, BIL3_silpo, BIL4_silpo, BIL5_silpo, BIL6_silpo, BIL7_silpo, BIL8_silpo, BIL9_silpo, BIL10_silpo, BIL11_silpo, BIL12_silpo, BIL13_silpo, BIL14_silpo, BIL15_silpo, BIL16_silpo, Bil1_rhile, BIL1_unknwn, and BIL2_unknwn.

113. (Withdrawn) The method of claim 106, wherein said autoprocessing segment is derived from a protein of an organism belonging to a genus selected from the group consisting of *Brucella*, *Clostridium*, *Magnetospirillum*, *Mannheimia*, *Methylobacterium*, *Neisseria*, *Pseudomonas*, *Rhodobacter*, *Silicibacter*, *Streptomyces*, *Thermobifida*, *Rhizobium*, *Chromobacterium*, *Myxococcus*, *Leptospira*, *Pirellula*, *Gemmata*, *Gloeobacter* and *Verrucomicrobium*.

114. (Withdrawn) The method of claim 113, wherein said organism is selected from the group consisting of *Rhodobacter capsulatus*, *Rhodobacter sphaeroides*, *Silicibacter pomeroyi*, *Brucella melitensis*, *Brucella suis*, *Magnetospirillum magnetotacticum*, *Methylobacterium extorquens*, *Rhizobium leguminosarum*, *Neisseria meningitidis*, *Neisseria meningitidis*, *Neisseria*

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meningitidis, Neisseria gonorrhoeae, Chromobacterium violaceum, Pseudomonas syringae, Pseudomonas fluorescens, Pseudomonas fluorescens, Mannheimia haemolytica, Myxococcus xanthus, Leptospira interrogans, Streptomyces coelicolor, Streptomyces avermitilis, Thermobifida fusca, Clostridium thermocellum, Pirellula species 1, Gemmata obscuriglobus, Gloeobacter violaceus, and Verrucomicrobium spinosum.

115. (Withdrawn) The method of claim 106, wherein said auto-cleavage results in removal of a segment of said chimeric polypeptide adjacent to an amino terminal end or a carboxy terminal end of said autoprocessing segment.

116. (Withdrawn) The method of claim 115, wherein said segment of said chimeric polypeptide adjacent to said autoprocessing segment is an amino terminal segment or a carboxy terminal segment of said chimeric polypeptide.

117. (Withdrawn) The method of claim 115, wherein said segment of said chimeric polypeptide adjacent to said carboxy terminal end of said autoprocessing segment includes an amino acid residue comprising a nucleophilic group at an amino terminal end thereof.

118. (Withdrawn) The method of claim 117, wherein said nucleophilic group is a hydroxyl group.

119. (Withdrawn) The method of claim 117, wherein said amino acid residue is a threonine residue.

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120. (Withdrawn) The method of claim 115, wherein said segment of said chimeric polypeptide adjacent to said amino terminal end of said autoprocessing segment includes a serine amino acid residue at a carboxy terminal end thereof.

121. (Withdrawn) The method of claim 106, wherein said chimeric polypeptide is capable of said auto-cleavage under a condition selected from the group consisting of a temperature selected from a range of 33 °C to 41 °C, a pH selected from a range of pH 7.8 to pH 8.2, and a concentration of dithiothreitol selected from a range of 0.1 mM to 20 mM.